Yeast peroxisomes

van der Klei, IJ; Veenhuis, M

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Yeast peroxisomes: function and biogenesis of a versatile cell organelle

Ida J. van der Klei and Marten Veenhuis

Yeast peroxisomes harbour enzymes involved in the metabolism of specific growth substrates. Sequestration of these enzymes increases the efficiency of such pathways. Currently, 16 genes involved in peroxisome biogenesis have been identified, and analysis of their products suggests novel mechanisms for organelle assembly and protein translocation.

I.J. van der Klei and M. Veenhuis are in the Dept of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Biological Centre, Kerklaan 30, 9751NN Haren, The Netherlands. *tel: +31 50 363 2176, fax: +31 50 363 5205, e-mail: ijvdklei@biol.rug.nl*

Microbodies (peroxisomes, glyoxysomes and glycosomes) represent a class of ubiquitous and important cell organelles, which are characterized by a proteinaceous matrix surrounded by a single membrane. Their physiological role is complex and variable, ranging from photorespiration in plant leaves to ether-lipid biosynthesis in mammalian cells[1]. In yeasts, microbody-bound enzymes are crucial for the metabolism of specific growth substrates (Table 1). In spite of this physiological diversity, the molecular mechanisms involved in the biogenesis of microbodies are highly conserved throughout the eukaryotic kingdom[2]. Microbodies are further classified on their physiological function. They are designated peroxisomes when they contain hydrogen peroxide-producing oxidases and are termed glyoxysomes when they harbour enzymes of the glyoxylate cycle. However, for simplicity, we will generally use the term peroxisome.

During the past decade, research on yeast peroxisomes has made major steps forward. An important milestone was the ability to induce peroxisome formation in *Saccharomyces cerevisiae* (1987)[3], which allowed application of the powerful molecular genetic techniques available for this organism. Simultaneously, molecular genetic techniques were developed for non-conventional yeasts, whose peroxisomes have been extensively studied since the early 1970s. These, and other, developments resulted in the isolation of peroxisome-deficient yeast mutants (*pex* mutants) in 1989 (Refs 4, 5) and cloning of the corresponding genes (*PEX* genes)[6]. Yeasts are now the model organisms of choice to study peroxisomes.

Peroxisome proliferation

In yeast, the number, size and enzyme content of peroxisomes is largely prescribed by the prevailing growth conditions. Cells grown on rich complex media contain one or only a few small peroxisomes. These organelles have been cytochemically characterized[7], but their physiological role is still unknown. As *pex* mutants grow at normal growth rates on rich media, the organelles are not essential. However, compared with wild-type cultures, slightly reduced yields are obtained, which suggests that compartmentation of peroxisomal enzymes within the organelle is favourable for growth. An alternative explanation for maintaining the 'rudimentary' peroxisomes during yeast growth on rich media originates in kinetic studies that have shown that these organelles serve as a target for newly synthesized peroxisomal enzymes after shifting cells to peroxisome-inducing media. As a result, the organelles grow and, at a certain size, multiply by division. Remarkably, after fission the mature organelle loses its capacity to incorporate additional proteins: protein import is confined to the smaller 'daughter' organelles that have budded off (Fig. 1). When cells are shifted from peroxisome-inducing conditions to media in which peroxisomes become redundant, the organelles are selectively degraded by an autophagic process. However, in each cell at least one small peroxisome is not degraded. Therefore, the advantage of retaining peroxisomes may lie in the ability to adapt rapidly to new growth conditions.

Recently, we proposed a hypothetical model to explain the heterogeneity of peroxisomes within yeast cells with respect to their capacity to import proteins and their sensitivity towards selective degradation (Fig. 1)[8]. According to this model, specific proteins involved in peroxisome biogenesis (peroxins[6]) form functional complexes, which are essential for peroxisomal protein import and membrane biogenesis. Functional complexes are mainly present in developing organelles, whereas they may be absent or inactivated in mature organelles. One explanation for the accumulation of functional complexes in newly formed organelles is that they are specifically donated to the developing organelle. Once the new organelle is formed, the 'mother organelle' lacks these complexes and loses its developmental functions but remains metabolically active (Fig. 1).

Two *PEX* genes function directly in peroxisome multiplication: *PEX10* from *Hansenula polymorpha*[9] and *PEX11* from *S. cerevisiae*[10,11] (Table 2). Overexpression of these genes, which both encode peroxisomal membrane proteins, results in the formation of increased numbers of relatively small organelles. In an *H. polymorpha PEX10* deletion strain, recognizable peroxisomal structures are absent, suggesting that both

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Table 1. Growth substrates shown to induce peroxisomes in yeasts, and the corresponding peroxisomal enzymes

Growth substrate	Microorganism	Peroxisomal enzymes
n-Alkanes	*Candida tropicalis*	Acyl-CoA oxidase, catalase, 2-enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, acetyl-CoA acyltransferase (thiolase), malate synthase, isocitrate lyase, isocitrate dehydrogenase ($NADP^+$), carnitine acetyltransferase.
D-Amino acids (carbon source)	*Candida boidinii* *C. tropicalis* *Candida utilis* *Pichia pastoris*	D-Amino acid oxidase, catalase.
D-Amino acids (nitrogen source)	*C. boidinii* *C. tropicalis* *C. utilis* *Hansenula polymorpha* *P. pastoris*	D-Amino acid oxidase, catalase.
Ethanol	*C. utilis* *H. polymorpha*	Isocitrate lyase, malate synthase, malate dehydrogenase, aspartate aminotransferase, glutamate dehydrogenase (NAD^+).
Ethylamine (carbon source)	*Trichosporon cutaneum*	Amine oxidase, catalase, isocitrate lyase, malate synthase, malate dehydrogenase, aspartate aminotransferase, glutamate dehydrogenase (NAD^+).
Fatty acids	*Saccharomyces cerevisiae* *P. pastoris* *Yarrowia lipolytica*	Acyl-CoA oxidase, catalase, 2-enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, acetyl-CoA acyltransferase (thiolase), malate synthase, isocitrate lyase[a], carnitine acetyl transferase, malate dehydrogenase, citrate synthase[b].
Methanol	*C. boidinii* *H. polymorpha* *P. pastoris* *Pichia methanolica* *Pichia pinus*	Alcohol oxidase, catalase, formaldehyde transketolase (dihydroxyacetone synthase).
Primary amines (nitrogen source)	*C. utilis* *H. polymorpha* *T. cutaneum*	Amine oxidase, catalase.
Urate (carbon source)	*Candida famata*	Urate oxidase, catalase.
Urate (nitrogen source)	*C. famata* *C. utilis* *H. polymorpha* *T. cutaneum*	Urate oxidase, catalase.

[a]Not found in *S. cerevisiae*.
[b]Only found in *S. cerevisiae*.

division and growth of the organelle are prevented. In contrast, deletion of *PEX11* in *S. cerevisiae* results in the formation of one or two large peroxisomes per cell, which implies that only organelle division is affected. Recently, Goodman and co-workers showed that in the smaller 'immature' organelles, Pex11p is present as a monomeric protein, whereas in larger 'mature' organelles, Pex11p homodimers are formed[12]. Interestingly, when dimerization of Pex11p is prevented (by a point mutation in *PEX11*), the number of organelles in the cells is increased[12]. Thus, dimeric Pex11p may prevent further import and budding of the organelles and cause organelle maturation. Possibly, differences in the oligomeric state of Pex11p may contribute to the heterogeneity of peroxisomes (Fig. 1).

Physiological functions of peroxisomes

Fundamentally, yeast peroxisomes can be described as 'enzyme bags'. Characteristic features of the organelles are their very high protein content and low surface : volume ratio. The latter may be related to the fact that peroxisomal enzymes exist primarily in the matrix, not in the membrane. In addition, the overall protein content of peroxisomal membranes is relatively low. The low abundance of large integral membrane proteins is also illustrated by the typically smooth fracture faces of peroxisomal membranes in freeze-etch replicas (Fig. 2).

Common enzymes involved in microbody metabolism in yeasts are those involved in hydrogen peroxide production and decomposition, the glyoxylate cycle and β-oxidation (Table 1). There is now ample evidence

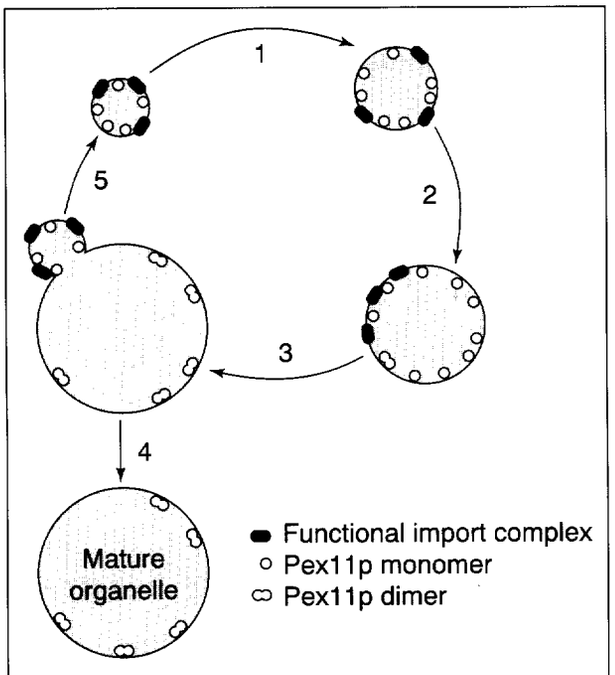

Fig. 1. Hypothetical model explaining peroxisome heterogeneity with respect to protein import and organelle maturation. Small immature peroxisomes are capable of incorporating newly synthesized proteins (1). The organelle grows (2,3) until a certain size is reached. At this stage, a new organelle buds off, resulting in a large mature organelle (4) and a newly formed, protein import-competent organelle (5). The mature organelle has lost the putative functional complexes required for protein import, which have been donated to the developing organelle. Maturation of peroxisomes is paralleled by Pex11p dimerization (3), which may influence the capacity of the organelles to grow[12].

that the general advantage of sequestering these enzymes in peroxisomes is that it increases the efficiency of specific metabolic pathways (e.g. methanol or ethanol metabolism and β-oxidation; Table 1). It must be emphasized that these metabolic pathways also function in *pex* mutants[13–15]. In these mutants, peroxisomal enzymes are normally synthesized and active but are located in the cytosol. However, this location has severe energetic or metabolic disadvantages, which prevent normal growth on the substrates that are metabolized by peroxisome-bound enzymes[13–15].

With respect to the metabolism of C2 compounds (ethanol/acetate), a remarkable discrepancy exists between the data obtained from *S. cerevisiae* and those from other yeasts. In non-conventional yeasts grown on C2 compounds, fatty acids or n-alkanes, the enzymes of the glyoxylate cycle [isocitrate lyase (ICL), malate synthase (MS) and malate dehydrogenase (MDH)] are found in peroxisomes (Table 1). In *H. polymorpha* and *Trichosporon cutaneum*, aspartate aminotransferase [AAT; also designated glutamate–oxaloacetate aminotransferase (GOT)] and glutamate dehydrogenase (NAD^+) activities are also associated with peroxisomes. These enzymes allow continuous oxidation of NADH produced by MDH (Fig. 3a)[16].

The location of the glyoxylate cycle, which is firmly established for non-conventional yeasts (see Table 1), has not yet been demonstrated unequivocally for *S. cerevisiae*, in which ICL activity is only found in cytosolic fractions[17,18]. Moreover, studies on constructed *S. cerevisiae* mutants have revealed that the location of peroxisomal MS in the cytosol[19] or the absence of peroxisomal MDH (Ref. 20) do not inhibit growth on C2 compounds. In contrast, deletion of the gene encoding peroxisomal MDH abolishes cell growth on oleic acid. Van Roermund *et al.*[20] suggest that under these conditions MDH is required for the oxidation of NADH produced by β-oxidation (Fig. 3b). This implies that, in *S. cerevisiae*, peroxisomal MDH would catalyse the reverse reaction to that required for the glyoxylate cycle. In addition, Elgersma and Tabak[21] have recently proposed that in *S. cerevisiae* the putative peroxisomal AAT, encoded by *AAT2*, does not generate aspartate as previously suggested[16] but catalyses the reverse reaction, namely production of oxaloacetate and glutamate from aspartate and 3-ketoglutarate. In this respect, AAT and MDH could function in a malate–aspartate shuttle to transport reducing equivalents across the peroxisomal membrane, a process that would require a 3-ketoglutarate/malate and a glutamate/aspartate carrier (Fig. 3b)[21]. *S. cerevisiae* probably represents an exception to the general rule that the glyoxylate cycle is peroxisome-bound, because, in contrast to other yeasts, it is capable of producing ethanol and, consequently, is less adapted to efficient C2 catabolism.

The peroxisomal membrane as a barrier

In vivo, the peroxisomal membrane is not permeable to small solutes. The presence of a pH gradient across the membrane necessarily implies that it is impermeable to protons[22]. As indicated above, NAD(H) probably cannot pass freely across the peroxisomal membrane. Moreover, acetyl CoA may only cross this barrier after conversion into intermediates of the glyoxylate cycle or as a carnitine ester[20]. This suggests the presence of several transporter proteins. So far, only two peroxisomal transporters have been identified in yeasts, namely PMP47 in *Candida boidinii*[23] and a member of the ABC (ATP-binding cassette) family of transporters in *S. cerevisiae*[24,25]. PMP47 of *C. boidinii* is homologous to proteins belonging to the mitochondrial family of solute transporters. Surprisingly, disruption of the gene encoding PMP47 results in a specific protein import defect for the peroxisomal enzyme formaldehyde transketolase (generally referred to as dihydroxyacetone synthase), which accumulates as protein aggregates in the cytosol[26]. A possible explanation is that PMP47 is involved in the transport of thiamine pyrophosphate (TPP), the cofactor of formaldehyde transketolase, into the organelle. Recently, Evers *et al.*[27] showed that the import and assembly of alcohol oxidase (AO) in peroxisomes of *H. polymorpha* are dependent on the availability of the cofactor flavin adenine dinucleotide (FAD). When FAD is limiting, the assembly of AO into active octamers is affected, resulting in the accumulation of inactive, monomeric AO protein in the cytosol[27]. Similarly, the presence of TPP inside peroxisomes may facilitate the import and assembly of formaldehyde transketolase.

In *S. cerevisiae*, a peroxisomal protein has been identified that is a member of the ABC family of transporters.

Table 2. Yeast genes involved in peroxisome biogenesis (*PEX* genes)[6,32]

Gene	Protein product	Putative function
PEX1	117–127 kDa; belongs to the family of AAA-ATPases.	?
PEX2	35–52 kDa; contains C_3HC_4 zinc finger motif; integral peroxisomal membrane protein.	?
PEX3	51–52 kDa; integral peroxisomal membrane protein.	?
PEX4	21–24 kDa; ubiquitin-conjugating protein; associated with the peroxisomal membrane.	?
PEX5	64–69 kDa; contains TPR motifs; found in cytosol, peroxisomal membrane and matrix.	Receptor of PTS1.
PEX6	112–127 kDa; belongs to the family of AAA-ATPases; cytosolic protein.	?
PEX7	42 kDa; contains seven WD40 motifs; found in cytosol, peroxisomal membrane and matrix.	Receptor of PTS2.
PEX8	71–81 kDa; contains PTS1 and PTS2; peroxisomal matrix protein.	?
PEX9	42 kDa; integral peroxisomal membrane protein.	?
PEX10	34–48 kDa; integral peroxisomal membrane protein; contains C_3HC_4 zinc finger motif.	Involved in peroxisome proliferation.
PEX11	27–32 kDa; peroxisomal membrane protein.	Involved in peroxisome proliferation.
PEX12	31 kDa; contains two putative C_3HC_4 zinc finger motifs.	?
PEX13	43 kDa; carboxy-terminal SH3 domain.	Component of receptor docking site.
PEX14	40 kDa; peroxisomal membrane-associated protein.	Component of receptor docking site.
PEX15	43 kDa; (formerly *PAS21* in *Saccharomyces cerevisiae*) peroxisomal integral membrane protein.	?
PEX16	44 kDa; matrix face of the peroxisomal membrane[53].	?

Abbreviations: AAA, ATPases associated with diverse cellular activities; PTS, peroxisomal-targeting signal; SH3 domain, Src-homology 3 domain; TPR, tetratricopeptide repeat.

It is a heterodimer of the gene products of *PXA1* and *PXA2* and is probably involved in transport of substrates for β-oxidation[24,25].

Biochemical evidence also exists for a calcium-regulated pore-forming protein and an H^+-ATPase in the peroxisomal membrane of *H. polymorpha*. Further analysis of these proteins awaits the cloning of the corresponding genes[22].

Peroxisome biogenesis

The original model of peroxisome biogenesis proposed that the organelles develop by budding from the endoplasmic reticulum (ER)[28]. This view changed with the discovery that peroxisomal membrane and matrix proteins are encoded by nuclear genes and synthesized on free ribosomes in the cytosol. The current model predicts that peroxisomes develop by fission from pre-existing ones and grow by post-translational import of membrane and matrix proteins[29].

The molecular mechanisms involved in sorting these proteins do not share the typical features of other extensively studied protein translocation mechanisms (e.g. for mitochondria, ER and secretion in bacteria). The most striking example is the finding that proteins to be incorporated into the organelle do not necessarily have to be unfolded (see below). Moreover, all the proteins involved in peroxisomal protein import that have been identified so far are novel proteins, which have no homologous counterparts in other organelles (Table 2). Hence, peroxisomal protein import seems to comprise novel and unique principles.

Matrix proteins are targeted by peroxisomal-targeting signals (PTS), which are present within the primary sequence of the proteins, either at the extreme carboxyl terminus (PTS1) or within the amino terminus (PTS2). So far, little is known concerning the sequences required for targeting of peroxisomal membrane

Fig. 2. Freeze-etch replica of *Hansenula polymorpha* grown on methanol. The smooth fracture faces, which are typical of peroxisomal membranes, suggest that the abundance of large integral membrane proteins is low. Abbreviations: ER, endoplasmic reticulum; P, peroxisome; M, mitochondrion; V, vacuole. Scale bar = 0.5 μm.

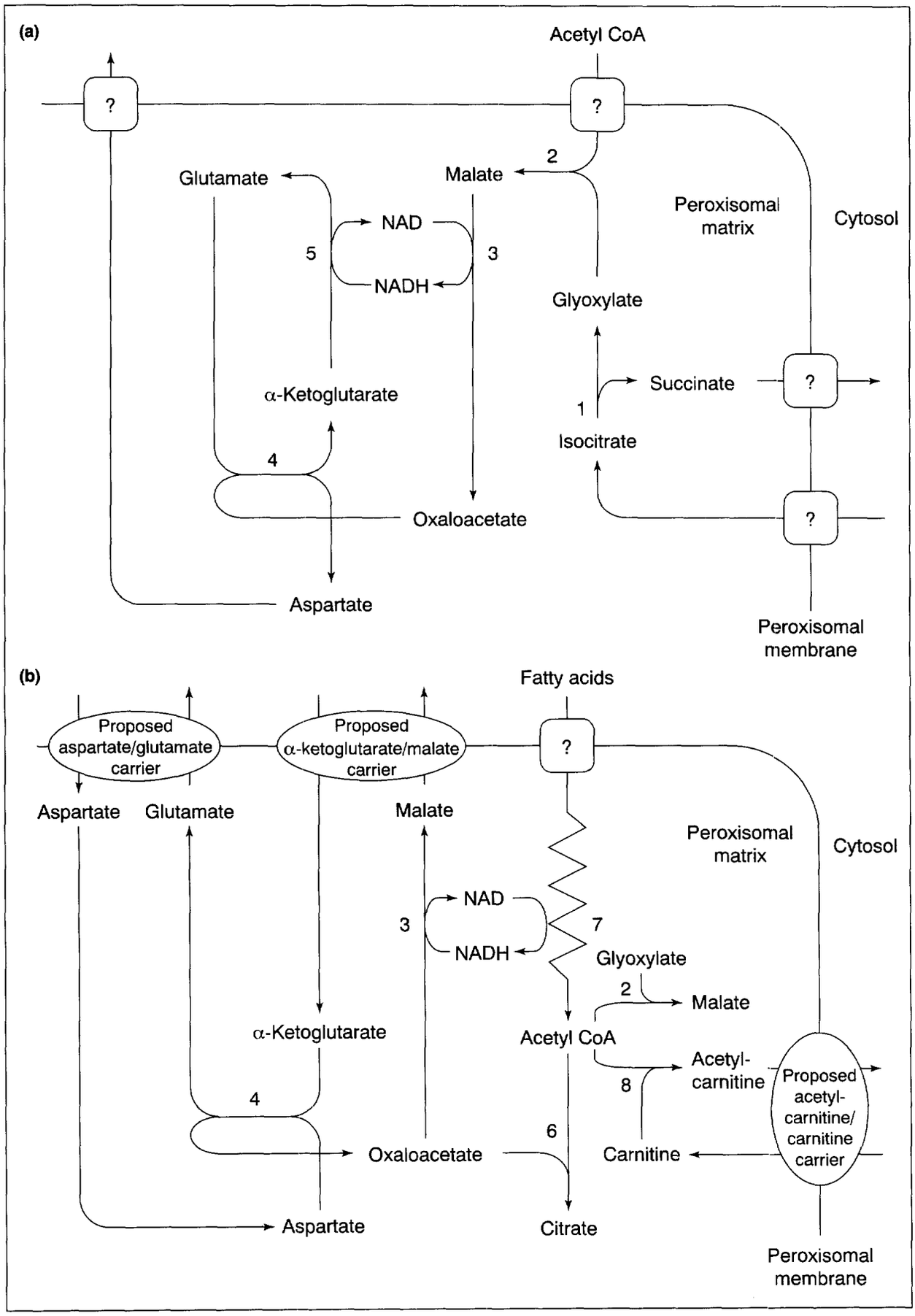
(a)
Acetyl CoA
?
?
2
Glutamate
Malate
Peroxisomal matrix
Cytosol
NAD
5
3
NADH
Glyoxylate
α-Ketoglutarate
Succinate
?
1
Isocitrate
4
Oxaloacetate
?
Aspartate
Peroxisomal membrane
(b)
Fatty acids
Proposed aspartate/glutamate carrier
Proposed α-ketoglutarate/malate carrier
?
Aspartate
Glutamate
Malate
Peroxisomal matrix
Cytosol
NAD
3
7
NADH
Glyoxylate
2
Malate
α-Ketoglutarate
Acetyl CoA
Acetyl-carnitine
8
Proposed acetyl-carnitine/ carnitine carrier
4
6
Oxaloacetate
Carnitine
Aspartate
Citrate
Peroxisomal membrane

Fig. 3. (facing page) Schematic representation of **(a)** the glyoxylate cycle, as proposed for non-conventional yeasts, and **(b)** peroxisome-bound metabolic pathways thought to occur in oleic acid-grown *Saccharomyces cerevisiae*. The enzymes catalysing each step are numbered: (1) isocitrate lyase; (2) malate synthase; (3) malate dehydrogenase; (4) aspartate aminotransferase; (5) glutamate dehydrogenase (NAD^+); (6) citrate synthase; (7) β-oxidation enzymes and (8) carnitine acetyltransferase. Boxes with question marks represent unidentified transporters (e.g. dicarboxylate and tricarboxylate carriers).

proteins (PMPs). Goodman and co-workers have identified a short hydrophilic loop in *C. boidinii* PMP47 (amino acids 225–244), which has been shown to contain peroxisomal targeting information[30]. Sequence comparisons have revealed a similar region in several other PMPs, including *H. polymorpha* Pex3p (amino acids 36–65). However, this region is not necessary for targeting of Pex3p to peroxisomes because the first 35 amino-terminal amino acids of Pex3p are also capable of targeting a reporter protein to the organelle[31].

Receptors shuttle between the cytosol and the peroxisome

The characterization of *PEX* gene products (peroxins) has resulted in the identification of receptor proteins for PTS1 (Pex5p) and PTS2 (Pex7p). By similar approaches, proteins involved in docking of the receptors at the peroxisomal membrane (Pex13p and Pex14p) have also been found (Table 2).

Conflicting data have been published with respect to the location of Pex5p and Pex7p. These vary between an exclusively cytosolic location, a membrane-bound location, a matrix location and a dual location in both the organelle and the cytosol. Despite this confusion, a widely accepted view is that both receptors bind newly synthesized matrix proteins in the cytosol and are recognized by peroxins [Pex13p (Refs 32–34) and Pex14p (Refs 35,36)] on the peroxisomal membrane. After delivery of their cargo, the receptors shuttle back to the cytosol. Whether the cargo dissociates from the receptors at the membrane or inside the matrix[37,38] is still a matter of debate (for reviews, see Refs 39,40).

At first glance, co-import of both the receptor and the cargo protein into the peroxisomal matrix may appear rather unusual. However, recent experiments have revealed that oligomerization of peroxisomal enzymes may actually precede the import process[41,42]. Thus, peroxisomal proteins do not necessarily have to be unfolded during translocation across the peroxisomal membrane. However, the mechanism for importing large, folded structures remains an enigma. Large pores, like those in the nuclear envelope, have never been detected, but the temporary formation of such pores cannot be excluded. Alternatively, proteins could be incorporated by membrane invaginations[41] or, eventually, during fusion of membrane vesicles with the peroxisomal membrane (see below), which may cause a temporary destabilization of the membrane.

Recent studies on *H. polymorpha* Pex4p, a ubiquitin-conjugating enzyme, suggest that it is required for recycling of Pex5p. In a *PEX4* null mutant, matrix protein import is highly reduced but can largely be restored by overproduction of Pex5p. The reasons behind this phenomenon are not yet clear. One plausible explanation is that modification of a protein by ubiquitination is an essential step for shuttling Pex5p back to the cytosol and is thus prevented in a *PEX4* null mutant. Overproduced Pex5p could then replenish the Pex5p trapped in peroxisomes after import. Alternatively, Pex4p could be essential to maintain functional import complexes. In this scenario, Pex4p carries out the classical function of ubiquitin-conjugating enzymes, namely tagging of proteins to be degraded, and ubiquitinates nonfunctional protein import complexes or individual components of this complex, which are subsequently degraded (as in the degradation of nonfunctional SecY complexes by FtsH in *Escherichia coli*[43]). In the absence of Pex4p, Pex5p may become trapped in nonfunctional protein complexes.

An alternative view of the function of the PTS receptors, based on the exclusively peroxisomal matrix location of *Yarrowia lipolytica* Pex5p observed by Rachubinski and co-workers[44] and that of *S. cerevisiae* Pex7p observed by Zhang and Lazarow[45], is that they 'pull' PTS-containing proteins into the peroxisomal matrix.

Can peroxisomes be formed de novo?

The current view of peroxisome biogenesis predicts that peroxisomes are formed from pre-existing organelles[29]. However, in *pex* mutants such organelles are absent, although most of them do contain remnant peroxisomal membrane structures, which harbour a minor portion of the matrix proteins or are empty ('ghosts')[46,47]. After re-introduction of the complementing gene, these structures may be used as a template to form a new organelle. However, there are also *pex* mutants in which these peroxisomal membrane remnants are undetectable[31,47,48]. These mutants are probably affected in one of the crucial steps in peroxisomal membrane biosynthesis. In addition, their peroxisomes readily reappear when the corresponding genes are re-introduced, implying that *de novo* synthesis of peroxisomes may be possible[48].

Because of the dogma that membranes have to arise from membranes, a major question is the origin of the newly formed organelles. It is still not known how the peroxisomal membrane is formed in wild-type cells. One possibility is that the ER and transport vesicles are involved. This is suggested by the finding that Pex1p and Pex6p, which are members of the AAA (ATPases associated with diverse cellular activities) protein family and are homologous to proteins involved in membrane fusion processes, are essential for peroxisome biogenesis[6] (Table 2). Hence, Pex1p and Pex6p may catalyse the fusion of vesicles with peroxisomal membranes. In addition, we have recently found that brefeldin A, a fungal toxin that prevents the formation of coated vesicles, affects the sorting of peroxisomal proteins in *H. polymorpha*[49].

Questions for future research

- How do peroxisomes function? Despite the elegant genetic studies by Tabak and co-workers[20,21], which predicted that various transporters/carriers must exist in the peroxisomal membrane, nothing is yet known concerning the mechanisms of solute transport across the peroxisomal membrane.
- What determines the remarkable heterogeneity between peroxisomes, with respect to peroxisomal protein import and degradation? Is import machinery in mature peroxisomes inactivated or, alternatively, donated to new developing organelles during the fission process?
- How does the protein import machinery function? Which proteins are involved, and does the endoplasmic reticulum play a role?
- Are oligomeric peroxisomal proteins imported by the same apparatus as 'normal' protein import, or is import coincidental, resulting from a side effect of peroxisome–vesicle fusion processes?
- Does the presumed peroxisomal protein export machinery, predicted from the shuttling of the peroxisomal-targeting signal receptors, exist as separate machinery or does it have elements in common with the import machinery?
- Can peroxisomes be formed *de novo* after re-introduction of the *PEX3* gene, as may be predicted from the rapid re-assembly of peroxisomes in Δ*pex3* strains, which lack peroxisomal membrane remnants?

On the basis of these findings, we propose that specific peroxisomal membrane proteins are first targeted to the ER and subsequently sorted to peroxisomes by a process that may involve vesicle transport and vesicle fusion events. This mode of development could explain why overproduction of specific peroxisomal membrane proteins, namely Pex3p (Ref. 31) and Pex14p (Ref. 35), results in the accumulation of these proteins on ER-like structures and why a truncated form of *S. cerevisiae* Pas21p is located in the cell membrane (Y. Elgersma, PhD thesis, Amsterdam, 1995). Possibly, the deleted region of Pas21p is required to prevent the protein from entering the secretory pathway.

Current questions

Is the ER involved in peroxisome biogenesis?

The current data on peroxisomes support the notion that they are essentially bags filled with enzymes. For instance, >90% of the total protein content of peroxisomes in methanol-limited *H. polymorpha* cells consists of the three major enzymes of methanol metabolism: alcohol oxidase, catalase and formaldehyde transketolase. This implies that <10% is taken up by other enzymes, peroxins and transporters in the peroxisomal membrane. The incorporation of additional matrix protein into this 'bag of enzymes' is likely to depend on a simultaneous increase in the surface area of the organelle membrane. This makes it tempting to speculate that the uptake of matrix proteins and membrane growth are coupled processes. As mentioned previously, specific integral peroxisomal membrane proteins may, after synthesis in the cytosol, be transported to the ER before they are delivered to the target peroxisome[49]. This ER–peroxisome pathway may involve vesicle trafficking and may, as a result of the fusion process, create a dynamic import site by bringing the various essential components together in the required functional stoichiometry[50]. Vesicle fusion processes may also account for the uptake of folded, oligomeric proteins by peroxisomes. Douma *et al.* have shown that fusion of empty liposomes with yeast protoplasts may, as a side effect, result in the simultaneous uptake of exogenously added oligomeric alcohol oxidase protein[51]. By analogy, uptake of complex proteins in peroxisomes may occur during peroxisome/vesicle fusion after selective delivery of the protein to a peroxisomal docking site.

How does the protein import complex function?

Both genetic (two-hybrid studies[36] and unlinked non-complementation[52]) and biochemical approaches (co-immune precipitations[36]) have established physical interactions between different peroxins, suggesting the presence of functional protein complexes. The individual protein components of the functional complexes are probably present at a rather strict stoichiometry that allows only minor modulation for proper functioning. Disturbance of this stoichiometry (e.g. by overproduction of one of the components, for example by overexpression of *PEX3* or *PEX14*) affects both protein import and normal formation of peroxisomes[31,35,50].

Detailed studies are required to identify the components of these complexes and to determine whether they are stable or dynamic. In addition, it is of utmost importance to develop reliable *in vitro* assays to test current models of peroxisomal protein translocation and membrane biogenesis. The ultimate goal is to reconstitute these processes *in vitro* using purified components.

How does the peroxisomal membrane function?

To understand the function of the peroxisomal membrane, analysis of its transport properties and the proteins involved is needed. One major problem associated with a biochemical approach to characterize these proteins is that peroxisomal membranes are leaky *in vitro*, probably as a result of the purification procedures[22]. Consequently, it is desirable to set up strategies to clone genes encoding peroxisomal proteins involved in solute transport. As mutants affected in these genes do not have a *pex* phenotype (Δ*pmp47*, Δ*pxa1* and Δ*pxa2*), novel mutant screens have to be designed.

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References

1 van den Bosch, H. *et al.* (1992) *Annu. Rev. Biochem.* 61, 157–197
2 Gould, S.J. *et al.* (1990) *EMBO J.* 9, 85–90
3 Veenhuis, M. *et al.* (1987) *Yeast* 3, 77–84
4 Erdmann, R. *et al.* (1989) *Proc. Natl. Acad. Sci. U. S. A.* 86, 5419–5423
5 Cregg, J.M. *et al.* (1990) *Yeast* 6, 87–97
6 Distel, B. *et al.* (1996) *J. Cell Biol.* 135, 1–3
7 Veenhuis, M. *et al.* (1979) *Arch. Microbiol.* 120, 167–175
8 van der Klei, I.J. and Veenhuis, M. (1996) *Ann. New York Acad. Sci.* 804, 47–59
9 Tan, X. (1995) *J. Cell Biol.* 128, 307–319
10 Erdmann, R. (1995) *J. Cell Biol.* 128, 509–523
11 Marshall, P.A. *et al.* (1995) *J. Cell Biol.* 129, 345–355
12 Marshall, P.A. *et al.* (1996) *J. Cell Biol.* 135, 123–137

13 Sulter, G.J. *et al.* (1990) *Yeast* 6, 501–509
14 van der Klei, I.J., Harder, W. and Veenhuis, M. (1991) *Arch. Microbiol.* 156, 15–23
15 Sulter, G.J. *et al.* (1991) *FEMS Microbiol. Lett.* 82, 297–302
16 Veenhuis, M., van der Klei, I.J. and Harder, W. (1986) *Arch. Microbiol.* 145, 39–50
17 McCammon, M.T. *et al.* (1990) *J. Bacteriol.* 172, 5816–5827
18 Taylor, K.M. *et al.* (1996) *Biochem. J.* 319, 255–262
19 Brocard, C. *et al.* (1996) *Ann. New York Acad. Sci.* 804, 694–695
20 Van Roermund, C.W.T. *et al.* (1995) *EMBO J.* 14, 3480–3486
21 Elgersma, Y. and Tabak, H.F. (1996) *Biochim. Biophys. Acta* 1286, 269–283
22 Sulter, G.J., Harder, W. and Veenhuis, M. (1993) *FEMS Microbiol. Rev.* 11, 285–296
23 McCammon, M.T. *et al.* (1990) *J. Biol. Chem.* 265, 20098–20105
24 Shani, N. and Valle, D. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 11901–11906
25 Hettema, E.H. *et al.* (1996) *EMBO J.* 15, 3813–3822
26 Sakai, Y. *et al.* (1996) *J. Cell Biol.* 134, 37–51
27 Evers, M.E. *et al.* (1994) *Mol. Biol. Cell* 5, 829–837
28 Novikoff, A.B. and Shin, W.H. (1964) *J. Microsc.* 3, 187–206
29 Lazarow, P.B. and Fujiki, Y. (1988) *Annu. Rev. Cell Biol.* 1, 489–530
30 Dyer, J.M., McNew, J.A. and Goodman, J.M. (1996) *J. Cell Biol.* 133, 269–280
31 Baerends, R.J.S. *et al.* (1996) *J. Biol. Chem.* 271, 8887–8894
32 Gould, S.J. *et al.* (1996) *J. Cell Biol.* 135, 85–95
33 Elgersma, Y. *et al.* (1996) *J. Cell Biol.* 135, 97–109
34 Erdmann, R. and Blobel, G. (1996) *J. Cell Biol.* 135, 111–121
35 Komori, M. *et al.* (1997) *EMBO J.* 16, 44–53
36 Albertini, M. *et al.* (1997) *Cell* 89, 83–92
37 van der Klei, I.J. *et al.* (1995) *J. Biol. Chem.* 270, 17229–17236
38 Dodt, G. and Gould, S.J. (1996) *J. Cell Biol.* 135, 1763–1774
39 Subramani, S. (1996) *J. Biol. Chem.* 271, 32483–32486
40 Erdmann, R., Veenhuis, M. and Kunau, W.H. (1997) *Trends Cell Biol.* 7, 400–407
41 McNew, J.A. and Goodman, J.M. (1994) *J. Cell Biol.* 127, 1245–1257
42 Glover, J.R. *et al.* (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 10541–10545
43 Akiyama, Y. *et al.* (1996) *J. Biol. Chem.* 271, 31196–31201
44 Szilard, K.R. *et al.* (1995) *J. Cell Biol.* 131, 1453–1469
45 Zhang, J.W. and Lazarow, P.B. (1996) *J. Cell Biol.* 132, 325–334
46 Purdue, P.E. and Lazarow, P.B. (1995) *Yeast* 11, 1045–1060
47 Veenhuis, M. *et al.* (1996) *FEBS Lett.* 383, 114–118
48 Waterham, H.R.W. *et al.* (1993) *EMBO J.* 12, 4785–4794
49 Salomons, F.A. *et al.* (1997) *FEBS Lett.* 411, 133–139
50 Baerends, R.J.S. *et al. Yeast* (in press)
51 Douma, A.C. *et al.* (1990) *Yeast* 6, 99–105
52 Titorenko, V.I. *et al.* (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 7470–7474
53 Eitzen, G.A. *et al.* (1997) *J. Cell Biol.* 137, 1265–1278

Dissecting the biology of a pathogen during infection

Douglas M. Heithoff, Christopher P. Conner and Michael J. Mahan

***In vivo* expression studies reveal many bacterial genes that contribute to the fitness of the organism in the context of host ecology. This collection of virulence genes defines the unique lifestyle of a pathogen during infection, pointing to the functions that dictate host specificity, tissue tropism and disease manifestation.**

D.M. Heithoff, C.P. Conner and M.J. Mahan are in the Dept of Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, CA 93106, USA.*
**tel: +1 805 893 7160, fax: +1 805 893 4724, e-mail: mahan@lifesci.lscf.ucsb.edu*

Microbial pathogenicity may be defined as the ability to enter into, replicate within, and persist at host sites that are inaccessible to commensal species[1]. These unique activities result in pathological lesions within the host, which, in turn, may lead to overt symptoms and disease. The standard assay to determine the involvement of a bacterial gene in the disease process is to mutate that gene and ascertain whether the mutation confers a virulence defect in an animal model. However, this is a low resolution assay because pathogenesis is multifactorial in nature and is not restricted to a single linear pathway from infection to mortality. For example, one of several redundant pathways may provide a subtle advantage, depending on the nutrition and/or fitness status of a given host, but mutations in individual genes within these pathways will often be ignored because LD_{50} studies may not detect their contributions.

Despite these difficulties, this virulence assay is thought by some to be the acid test for determining whether a gene encodes a virulence factor. It may be argued, however, that if a function contributes to the fitness of the organism *in vivo*, it may be defined as a virulence factor even though a mutation in that gene has no detectable phenotype in an LD_{50} assay. Thus, contributions to growth within the host and subsequent transmission to new hosts may have profound effects on the evolution of virulence. These subtle contributions may only be detected in alternative assays, such as the competitive index, in which mutant strains compete with the wild type within the host, or in more defined systems such as infected primary or immortalized cultured cells. Additionally, investigation of the expression patterns of many other virulence functions in well-characterized metabolic pathways (e.g. Mg^{2+} and Fe^{2+} transport) provides a

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